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(21) International Application Number: PCT/US95/15613 (22) International Filing Date: 12 December 1995 (12.12.95) (30) Priority Data: 08/357,121 15 December 1994 (15.12.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/357,121 (CIP) Filed on 15 December 1994 (15.12.94) (71) Applicants (for all designated States except US): THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). BOEHRINGER INGELHEIM KG [DE/DE]; Postfach 200, D-55216 Ingelheim am Rhein (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): HALL, Edward, Dallas [US/US]; 1432 Woodland Avenue, Portage, MI 49002 (US). VON VOIGTLANDER, Philip, F. [US/US]; 1 South Lake Doster, Plainwell, MI 49080 (US). ROHDE, Frank, A. [DE/DE]; Am Geistert 4, D-55413 Weiler (DE).		(74) Agent: WELCH, Lawrence, T.; The Upjohn Company, Corporate Intellectual Property Law, 301 Henrietta Street, Kalamazoo, MI 49001 (US). (81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: USE OF PRAMIPEXOLE AS A NEUROPROTECTIVE AGENT		
(57) Abstract <p>The present invention provides the use of pramipexole as a neuroprotective agent.</p>		

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USE OF PRAMIPEXOLE AS A NEUROPROTECTIVE AGENT

FIELD OF THE INVENTION

The present invention relates to the use of pramipexole or 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzo-thiazole, or the (+)- or (-)-enantiomers thereof,
5 and the pharmacologically acceptable salts thereof, as a neuroprotective agent.

BACKGROUND OF THE INVENTION

A number of central nervous system diseases and conditions result in neuronal damage. These conditions which can lead to nerve damage include:

Primary neurodegenerative disease; Huntington's Chorea; Stroke and other
10 hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Parkinson's disease; Alzheimer's disease, Senile Dementia of Alzheimer's Type (SDAT); age associated cognitive dysfunctions; or vascular dementia, multi-infarct dementia, Lewy body dementia, or
15 neurodegenerative dementia.

Pramipexole is a dopamine-D₃/D₂ agonist the synthesis of which is described in European Patent 186 087 and its counterpart, U.S. Patent 4,886,812. It is known primarily for the treatment of schizophrenia and Parkinson's disease. It is known from German patent application DE 38 43 227 that pramipexole lowers the plasma
20 level of prolactin. Also, this European patent application discloses the use of pramipexole in the treatment of drug dependency. Further, it is known from German patent application DE 39 33 738 that pramipexole can be used to decrease abnormal high levels of thyroid stimulating hormone (TSH). U.S. patent 5,112,842 discloses the transdermal administration of the compounds and transdermal systems
25 containing these active compounds. WO patent application PCT/EP 93/03389 describes the use of pramipexole as an antidepressant agent.

Up to now there is no commercially available drug for the therapeutic treatment of stroke with proven evidence of efficacy.

Surprisingly and unexpectedly, it has been found that pramipexole and its
30 (+)-enantiomer also has a neuroprotective effect.

INFORMATION DISCLOSURE

Piribedil, a vasodilator which binds to a multitude of receptors including dopamine receptors, is reported to have an effect on functional and biochemical parameters in a gerbil model of global cerebral ischemia. See, e.g., Society for
35 Neuroscience Abstracts, 19:673 (1993); *id.*, at 1645.

Lisuride binds to several different receptors including dopamine D₂ and 5-HT_{1a} receptors. It is reported that Lisuride, when administered before the event, reduced brain edema and prolonged survival time in a rat model of cerebral infarction. Miya Zawa, et al. Nippon-Yakurigaku-Zasshi 98(6):449-561, (1991).

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SUMMARY OF THE INVENTION

The present invention particularly provides a method for preventing neuronal damage in a patient suffering from or susceptible to such neuronal damage comprising the administration of an effective amount of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole, its (-)-enantiomer or (+)-enantiomer thereof, and
10 pharmacologically acceptable salts thereof especially an effective amount of pramipexole which is the (-)-enantiomer of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole-dihydrochloride or an effective amount of the (+)-enantiomer of 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole dihydrochloride.

15

Conditions which can cause nerve damage are well-known to an ordinarily skilled neurologist or similar physician and include:

- Primary neurogenerative disease;
- Huntington's Chorea;
- Stroke and other hypoxic or ischemic processes;
- 20 Neurotrauma;
- Metabolically induced neurological damage;
- Sequelae from cerebral seizures;
- Hemorrhagic stroke;
- Secondary neurodegenerative disease (metabolic or toxic);
- 25 Parkinson's disease;
- Alzheimer's disease, other memory disorders; or
- Vascular dementia, multi-infarct dementia, Lewy body dementia, or neurogenerative dementia.

The preferred indication for pramipexole, in the context of the present
30 invention, is Parkinson's disease which is characterized by progressive degeneration of nigrostriatal dopamine neurons. In this sense, the term Parkinson's disease also comprises the term Parkinson's syndrome. In addition to pramipexole's palliative action (i.e. replacement of the lost dopamine neurotransmitter function), the compound may slow the degeneration of surviving dopamine neurons and thereby
35 slows the progression of the disease.

The prophylactic use of the compound of this invention includes use as monotherapy in early or pre-symptomatic stages of Parkinson's disease and prevention of neurodegenerative disorders based on ischaemia.

The synthesis, formulation and administration of pramipexole is described in
5 U.S. patents 4,843,086; 4,886,812; and 5,112,842; which are incorporated by reference herein.

2-Amino-6-n-propyl-amino-4,5,6,7-tetrahydrobenzothiazole, particularly the (-)-enantiomer thereof, and the pharmacologically acceptable acid addition salts thereof can be given for preventing of neuronal damage. The form of conventional
10 galenic preparations consist essentially of an inert pharmaceutical carrier and an effective dose of the active substance; e.g., plain or coated tablets, capsules, lozenges, powders, solutions, suspensions, emulsions, syrups, suppositories, etc.

L-dopa is shown to be toxic to cerebellar granule cells in culture. Pramipexole and the (+) enantiomer blocked L-dopa toxicity. The EC₅₀ for both was
15 between 0.3 and 1 uM and 10 uM provided viability measurements that were equal to control cells not exposed to L-dopa. The mechanism of protection does not appear to involve receptor activation given that the (+) enantiomer is less active in monoamine receptor binding assays. The possibility exists that 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole, its (+) and (-) enantiomers and the
20 pharmacologically acceptable acid addition salts thereof, especially pramipexole and the (+) enantiomer are acting as antioxidants toward reactive oxygen species known to be generated from L-dopa incubation.

The effective dose range is 0.01 to 2.0 mg/kg. More preferred is a dose of 1-2 mg/kg PO. The preferred total dose level for neuroprotection is 0.5-20 mg/kg/day
25 PO. The preferred human dose is 0.1-6.0 mg/day total dose, divided in 2 or 3 administrations. In addition to being administered by oral or intravenous route, pramipexole may be administered transdermally.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is seen more fully by the examples given below:

30 Example 1:

We examined histologically retrograde degeneration of the dopaminergic nigrostriatal (NS) tract over 28 days in gerbils subjected to a 10-minute period of forebrain ischemia via bilateral carotid occlusion. There was in control animals a 39% loss of NS cell bodies in the zona compacta (as judged by tyroxine hydroxylase
35 immunocytochemistry) over that time period. Daily post-ischemic oral dosing (1

mg/kg PO BID beginning at 1 hr after insult) with pramipexole attenuated 28-day post-ischemic loss of NS DA neurons by 36% ($p < 0.01$ vs vehicle-treated).

Example 2:

We have successfully replicated the finding of Example 1; i.e., that
5 pramipexole can protect nigrostriatal dopamine neurons in a gerbil model of 10
minutes of bilateral carotid occlusion-induced forebrain ischemia plus 28 days of
post-ischemic survival. The administration of pramipexole was via an initial dose of
1 mg/kg PO 60 minutes after ischemia and again at the end of day 1 followed by
twice daily dosing for the next 26 days. A twice daily dose of 0.3 mg/kg produced a
10 threshold effect (14% increase in nigrostriatal dopamine neurons compared to
vehicle). A twice daily dose of 1 mg/kg produced a significant 38% improvement in
28 day post-ischemic dopamine neuronal survival ($p < 0.0001$ vs vehicle-treated).
This action appears to be specific for dopamine neurons since the post-ischemic loss
of the non-dopaminergic neurons in the CA1 area of the hippocampus was not
15 significantly affected.

Example 3:

Primary Cultures of Cerebellar Granular Cells

Primary cultures of cerebellar granular cells were prepared from 8-day-old
Sprague Dawley rats (Charles River, Portage, MI) as described previously (1).
20 Neurons were grown on 6-well, 35 mm culture dishes (Nunc, Denmark) for 8-9 days,
2 ml/well, at a density of 1×10^6 cells/ml. Glial cell proliferation was prevented by
adding cytosine-arabino-furanoside-monophosphate (Sigma, St. Louis, MO), 19 hrs
after plating at a final concentration of 10 μ M. Cultures generated by this method
have been characterized and shown to contain more than 90% granule cells (2).

25 ***Cell Toxicity Models***

Experiments were started after cells were 8 days in vitro (8 DIV). Cells were
washed with two ml of serum free growth medium. Concentrations of 100X stock
solutions of PPX and the (+) enantiomer in serum free growth medium were made.
These were delivered to cells by adding 20 μ l to cells in growth medium per well.
30 The final concentration of drugs ranged from 1 nM to 10 μ M. After 5 min 20 μ l of a
10X stock solution of L-dopa in serum free growth medium was added to each well
so that the final concentration was 100 μ M. Cells were incubated under the above
conditions for 24 hrs. For viability measurements cells were then washed twice and
pulsed with 1 μ Ci/ml of α -(methyl)- 14 C-aminoisobutyric acid (New England
35 Nuclear) in Locke's buffer for 1 hr. Cells were solubilized, following a wash, with

0.5% Triton X-100. The solubilized cells were then counted for radioactivity on a scintillation counter. Data was expressed as the mean of triplicates \pm S.D. for each point.

Results from the table show that pramipexole was neuroprotective toward the toxicity (64.8 %) associated with L-dopa. At 10 μ M pramipexole, L-dopa treated cells were not different from controls. Pramipexole is shown to decrease slightly but significantly cAMP levels in cerebellar granular cells (3). This suggests the possible involvement of dopamine receptors (D_2 family) in the mechanism of neuroprotection. To test this hypothesis, the (+) enantiomer was tested in a parallel experiment with pramipexole. The (+) enantiomer has been shown to be inactive in a battery of binding assays that involve adrenergic and serotonergic receptors, and less active in dopaminergic receptors. The results show that the (+) enantiomer is equally as potent and effective compared to pramipexole as a neuroprotective agent in this assay.

The neuroprotective effects of pramipexole in L-dopa mediated toxicity in cerebellar granule cell does not appear to involve the activation of dopamine receptors.

The (+) enantiomer of pramipexole shows utility as a neuroprotectant despite the fact that it shows little ability to bind to monoamine receptors.

The possibility exists that pramipexole and its (+) enantiomer can act as an antioxidant toward L-dopa toxicity which is known to involve the generation of reactive oxygen species.

TABLE 1
Toxicity (64.8%) of 100 μ M L-dopa:
Effects of pramipexole and (+) enantiomer.

	Dose (nM)	Pramipexole (% of control*)	(+) enantiomer (% of control*)
30	0	35.2 \pm 8.5	35.2 \pm 8.5
	1	30.0 \pm 6.5	51.3 \pm 5.8
	10	39.2 \pm 9.4	57.8 \pm 4.9
	100	64.8 \pm 4.7	58.3 \pm 10.3
	300	76.2 \pm 7.3	81.0 \pm 18.3
35	1000	65.5 \pm 14.8	86.9 \pm 7.0
	3000	84.9 \pm 0.6	95.2 \pm 2.5
	10000	90.0 \pm 11.5	99.8 \pm 8.9

* Control is defined as the radioactivity associated with cells that were exposed to vehicle buffer rather than 100 μ M L-dopa. Control value was equal to an average of 129547 CPMs for triplicate counts.

CLAIMS

1. A method for preventing neuronal damage or the progression of neuronal damage in a patient suffering from or susceptible to such neuronal damage
5 comprising the administration of an effective amount of the compound 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole and the pharmacologically acceptable acid addition salts thereof.
2. A method of claim 1 where the condition is selected from Parkinson's disease,
10 primary neurodegenerative disease; Huntington's Chorea; stroke and other hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Alzheimer's disease, other memory disorders; or vascular dementia, multi-infarct dementia, Lewy body dementia, or neurogenerative
15 dementia.
3. A method of claim 2, where the condition is Parkinson's disease.
4. A method of claim 3, where the total dose of the compound is about
20 0.1-6 mg/day.
5. A method of claims 1-4, wherein the compound is the form of the (+) enantiomer.
- 25 6. A method of claims 1-4, wherein the compound is the form of the (-) enantiomer.
7. A method of claims 1-6, wherein the compound is the dihydrochloride.
- 30 8. Use of a compound selected from 2-amino-6-n-propylamino-4,5,6,7-tetrahydrobenzothiazole and the pharmacologically acceptable acid addition salts thereof for preparing a medicament for preventing neuronal damage or the progression of neuronal damage.
- 35 9. A use of claim 8, wherein the neuronal damage results from Parkinson's

disease, primary neurodegenerative disease; Huntington's Chorea; stroke and other hypoxic or ischemic processes; neurotrauma; metabolically induced neurological damage; sequelae from cerebral seizures; hemorrhagic stroke; secondary neurodegenerative disease (metabolic or toxic); Alzheimer's disease, other memory disorders; or vascular dementia, multi-infarct dementia, Lewy body dementia, or neurodegenerative dementia.

10. A use of claim 8, wherein the neuronal damage results from Parkinson's disease.
- 10
11. A use of claims 8-10, wherein the dosage is 0.1-6 mg/day.
12. A use of claims 8-11, wherein the compound is the (+) enantiomer.
- 15 13. A use of claims 8-12, wherein the compound is the (-) enantiomer.
14. A use of claims 8-13, wherein the compound is the dihydrochloride.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/15613

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	EP,A,0 186 087 (KARL THOMAE GMBH) 2 July 1986 cited in the application see page 13 - page 19 ---	1-3, 5-10, 12-14
X	CURR. OPIN. NEUROL. NEUROSURG., vol. 6, no. 3, 1993 pages 339-343, XP 000568176 A. LIEBERMAN 'Treatment of Parkinson's disease.' see page 341 --- -/--	1-3,8-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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P,X	NEUROSCI. LETT., vol. 196, no. 1-2, 1995 pages 97-100, XP 000568217 M. CAMACHO-OCHOA ET AL. 'Rar brain binding sites for pramipexole, a clinically useful D3-preferring dopamine agonist.' see the whole document -----	1-3, 7-10,14

INTERNATIONAL SEARCH REPORT

Information on patent family members

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